Anti-arthritic activity of *Pogostemon quadrifolius*

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A B S T R A C T

Aim of the study: To investigate anti-arthritic activity of Aqueous methanolic extract of *Pogostemon quadrifolius* (AMEPQ) in Freund's complete adjuvant (FCA)-induced arthritis in rats.
Methods: The AMEPQ was prepared and subjected to acute oral toxicity in mice and tested against FCA induced arthritis in rats. Arthritis assessment was done by measuring – paw volume, joint diameter, pain threshold, thermal hyperalgesia, mechanical nociceptive threshold and body weight.
Haematological, serum, biochemical and in vivo anti-oxidant parameters were measured on the last day of the study. Histopathological and radiological analyses of ankle joints were also done. MEPQ was administered at the dose of 100, 200 and 400 mg/kg body weight.
Results: MEPQ dose dependently showed anti-arthritic activity which was evident with decrease in paw volume, joint diameter and increase in pain threshold, paw withdrawal latency, mechanical nociceptive threshold and body weight when compared to arthritic control group. AMEPQ (400 and 200 mg/kg) exhibits significant ($P < 0.001$ and $P < 0.01$, respectively) anti-arthritic activity by increasing levels of RBC, Hb and by decreasing levels of WBC, platelets and also serum C-reactive protein (CRP) and Rheumatoid factor (RF). The anti-arthritic activity was also confirmed with the altered biochemical parameters (AST, ALT, ALP and total protein level) and anti-oxidant parameters (SOD, MDA and GSH). MEPB (400 and 200 mg/kg) and diclofenac (10 mg/ kg) also inhibited joint destruction (histopathological and radiological analysis). *Conclusion: P. quadrifolius* may be a potential preventive or therapeutic candidate for the treatment of inflammation and arthritis.

1. Introduction

Arthritis and related disorders, including rheumatoid arthritis (RA), are common diseases affecting millions of people [1]. RA is characterized by articular injuries having an inflammatory propagation of synovial cells, attaining a nearly complete functional defect. It affects about 1% of the general population [2]. RA is a kind of chronic inflammatory autoimmune disease. Although a num-ber of drugs used in the treatment of RA have been developed over the past few decades, there is still a need for more effective drugs with lower side effects [3]. Conventional medicine, includ-ing treatment with steroids, nonsteroidal anti-inflammatory drugs (NSAIDs) and such biological agents as tumour necrosis factor alpha (TNF-*α*) and interleukin-1 beta (IL-1*β*) antagonists, has shown only limited success against RA. Such therapies are helpful in controlling the symptoms of acute RA, but their effects on chronic, prolonged RA are unsatisfactory. Moreover, the adverse effects of drug therapy are significant and include gastrointestinal disturbances, infections and cardiovascular risks [4].

Freund's complete adjuvant (FCA)-induced arthritis in rats has been employed widely as a model for chronic systemic inflam-mation and possesses many features in common with human rheumatoid arthritis [5]. FCA-induced arthritis is of considerable relevance for the study of pathophysiological and pharmacologi-cal control of inflammatory processes, as well as the evaluation of anti-arthritic effects of drugs [6]. The FCA-induced arthritis follows a biphasic time course, consisting of an acute local inflammatory reaction that subsides after 3–4 days and a chronic systemic reac-tion that shows a relapsing-remitting course after the initial two weeks and can persist for several months [7]. It is not known why this biphasic pattern of activity is seen but it may be due to an ini-tial stimulus caused by the injection of FCA followed by the delayed hypersensitivity response known to be induced by FCA [8].

Pogostemon quadrifolius. Fam. *Laminacea* is a rare existence Indian medicinal plant found in moist habitats such as along watercourses and in rice fields throughout most of peninsular and northern India at an elevation of 1300 m. *P. quadrifolius* is reported to have antimicro- bial, anthelmintic, hypotensive and stomach relieving properties [9]. *P. quadrifoliu*s used to treat inflammations and pulmonary tuberculosis [10] and also sesquiterpene lactones like santa- marine, 9 *13*-acetoxycostunolide and 9 *13*-acetoxyparthenolide isolated from *P. quadrifolius* exhibited significant anticancer activities in vitro [11]. Literature survey revealed the presence of chem- ical constituents like eudesmanolide, guaianolide, sesquiterpene lactones, isoivangustin and guaianolide [9], 6 *α*-hydroxy-4(14), 10(15)-guainadien-8*α*-, 12-olide [12]. Some sesquiterpene lac- tones isolated from other plants have been found to possess good anti-inflammatory activities [13].

As the literature survey shows that *P. quadrifolius* may show anti- inflammatory activity due to presence of sesquiterpene lactones and be utilized for treating pathological states like arthritis [14], therefore the objective of the present study was to determine the efficacy of *P. quadrifolius* (whole plant) in Freund's complete adjuvant (FCA)-induced arthritis in rats.

2. Materials and methods

2.1. Procurement and authentification of plant material

The leaves ,stem and flower part of Pogostemon quadrifolius Were collected from chinnaraavigudem reserve forest manuguru mandal bhadradri dist Telangana state .were collected from district ,Telangana ,india in the month of december 2019 and were preserved as herbarium specimen ,identified and authenticated by Plant taxonomist (IAAT : 337) K.Madhava chetty Dept of botany Sri venkateswara university ,Tirupathi Andhra pradesh

2.2. Drugs and chemicals

FCA (Sigma Aldrich, USA), diclofenac (gift sample from Emcure pharmaceuticals Ltd., Pune), methanol (Molychem, India), biochemical diagnostic kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and alkaline phosphatase (ALP) (Accurex biomedical Pvt. Ltd) were purchased. All other solvents and chemicals used for the study were of analytical grade and purchased from authentic vendors.

2.3. Experimental animals and approval

Female Wistar rats weighing (180–220 g) and female Swiss albino mice (25–30 g) were obtained from National Toxicology Centre, Pune, India. The animals were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12 h light: 12 h dark cycle. The experimental protocol was approved by Institu- tional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (PBCSEA), India (xxxxxxx).

2.4. Preparation of extract

The whole plant was shade dried and powdered. Dried powder (100 g) was subjected to aqueous methanol extraction by cold maceration with intermittent shaking and then filtered (yield 6.7% w/w). The extract was suspended in Tween-80 (0.1% v/v) to get the suspension for administration to rats [15].

2.5. Acute oral toxicity study

Healthy female Swiss albino mice were subjected to acute oral toxicity studies as per OECD guideline-425. The mice were fasted overnight, provided only with water. Methanol extract of *P. quadrifolius* (MEPQ) was administered orally at one dose level of 2000 mg/kg body weight. The mice were observed continuously for behavioural and autonomic profiles for 2 h and for any signs of toxicity or mortality up to 48h [16].

2.6. Freund's complete adjuvant-induced arthritis

Arthritis was induced by single intra-dermal injection of 0.1 mL of Freund's complete adjuvant into a footpad of the left hind paw of female rats. Each milliliter contains 1 mg of *Mycobacterium tuberculosis* (Strain H37Ra, ATCC-25177). The rats were anesthetized with ether inhalation during adjuvant injection, as the viscous nature of adjuvant exerts difficulty while injecting [17].

The animals were divided into six groups consisting of six animals per group:

- group I – healthy control;
- group II – arthritic control;
- group III – diclofenac 5 mg/kg, per os;
- group IV – MEPB 100 mg/kg, per os;
- group V – MEPB 200 mg/kg, per os;
- group VI – MEPB 400 mg/kg, per os.

Anti-arthritic activity of MEPB was evaluated on injected paw on the following parameters paw volume, joint diameter, pain threshold, thermal hyperalgesia, tactile allodynia and body weight on day 0, 1, 4, 8, 12, 16, 20, 24, and day 28 [18]. On day 28 the animals were anaesthetized with anaesthetic ether and the blood was withdrawn by retro-orbital puncture for the estimation of various biochemical parameters and haematological parameters.

2.6.1. Measurement of paw volume

Paw volume was measured using a Plethysmometer (UGO Basile, Italy) on day 0 before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28 [19]. The change in paw volume was calculated as the difference between the final and initial paw volume.

2.6.2. Measurement of joint diameter

Joint diameter was measured using a digital Vernier caliper (Mitutoyo, Japan) on day 0 before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28 [20]. The change in joint diameter was calculated as the difference between the final and initial joint diameter.

2.6.3. Measurement of pain threshold

Pain threshold was measured using Randall-Selitto analgesiometer (UGO Basile, Italy) on day 0 just before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28. The hind paw was placed between the flat surface and blunt pointer and applied increasing pressure. The cut-off pressure was 450 g. The pain threshold was determined when rat attempted to remove the hind paw from the apparatus [21].

2.6.4. Measurement of thermal hyperalgesia (paw withdrawal latency)

Thermal hyperalgesia of injected paw was measured using a radiant heat apparatus (UGO Basile, Italy) on day 0 just before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28. The paw was placed on the heat radiator with infrared intensity of

lamp was set at 40. A cut of latency of 15 s was used to avoid tissue damage [22].

2.6.5. Measurement of mechanical nociceptive threshold

Mechanical nociceptive threshold was determined by measuring paw withdrawal following probing of the plantar surface with a series of calibrated fine filaments (Von Frey hairs, Almemo, Germany) of increasing gauge [23,24]. The rats were allowed to acclimatize for 10 min in the Perspex box and Von Frey hairs (0.6 to 12.6 g) were applied to plantar surface of left hind paw. A series of three stimuli were applied to each paw for each hair within a period 2–3 s. The lowest weight of Von Frey hair to evoke a withdrawal from the three consecutive applications was considered to indicate the threshold. Lifting of the paw was recorded as a positive response [25].

2.6.6. Body weight recording

Body weight was recorded on day 0 just before FCA injections and thereafter on day 1, 4, 8, 12, 16, 20, 24, and day 28 [26].

2.6.7. Radiological analysis of ankle joints

On day 28, rats were anaesthetized and radiographs of the adjuvant injected hind paws were taken using X-ray (AGFA CR 30-X unit, Germany). Radiographic analysis of hind paws was performed at 55 kV peak, 50 mA and the exposure time was 5 s.

2.6.8. Haematological and serum parameters

On day 28, haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and platelets (PLT) were determined by usual standardized laboratory method [27]. Serum C-reactive protein (CRP) and Rheumatoid factor (RF) level was also measured [15].

2.6.9. Biochemical parameters

On day 28, blood of the rats was withdrawn by retro-orbital puncture and serum was used for the estimation of serum AST, ALT, ALP and total protein levels [27].

2.6.10. Anti-oxidant parameters

The rats were sacrificed on day 28 by cervical dislocation and liver of rats were isolated and washed in ice-cold saline. Tissue homogenates were prepared with 0.1 M tris-HCl buffer (pH 7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD), malonaldehyde (MDA), and reduced glutathione (GSH). SOD, MDA and GSH assay was performed as per the method of Misera and Fridocich 1972; Slater and Sawyer 1971; Morgon et al., 1979 [28–30] respectively.

2.6.11. Histopathological analysis of ankle joints

On day 28, ankle joints were separated from the hind paw and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5 μ thickness. The sections were stained with haematoxylin-eosin and evaluated under light microscope with 10 $\times$ magnifications for the presence of inflammatory cells, hyperplasia of synovium, pannus formation and destruction of joint space [31].

2.6.12. Measurement of spleen and thymus weight

On day 28, spleen and thymus of all the rats were removed and weighed [32].

2.7. Statistical analysis

Data was expressed as mean$\pm$SEM and statistical analysis was carried out by using GraphPad 5.0 software (GraphPad, San Diego, USA) by applying two-way ANOVA with Bonferroni test or one-way ANOVA with Dunnett's test. $P < 0.05$ was considered to be significant.

3. Results

3.1. Acute oral toxicity study

MEPQ did not exhibit any toxic symptoms and mortality when given orally at dose of 2000 mg/kg b.w. Therefore three doses (100, 200 and 400 mg/kg b.w) were selected for pharmacological studies.

3.2. Measurement of paw volume

There was significant ($P < 0.001$) increase in paw volume of all the rats treated with FCA compared to healthy control. MEPB (200 and 400 mg/kg) significantly ($P < 0.001$) lowered the paw volume from day 20 onwards as compared to FCA control group. MEPB lower dose 100 mg/kg was less effective, it significantly ($P < 0.01$) lowered paw volume on day 28. Diclofenac 10 mg/kg showed significant ($P < 0.001$) reduction in paw volume from day 16 onwards. The change in paw volume of MEPB treated (400 mg/kg; 1.42 $\pm$ 0.18 and 200 mg/kg; 2.13 $\pm$ 0.07) was evident as compared to FCA control (3.81 $\pm$ 0.10) on day 28 (Fig. 1).

3.3. Measurement of joint diameter

There was significant ($P < 0.001$) increase in joint diameter of rats of all the groups treated with FCA compared to healthy control. MEPQ (200 and 400 mg/kg) significantly ($P < 0.01$ and $P < 0.001$, respectively) decreased the joint diameter from day 20 as compared to FCA control. The change in joint diameter of MEPQ treated (400 mg/kg; 1.39 $\pm$ 0.19 and 200 mg/kg; 2.12 $\pm$ 0.06) was evident as compared to FCA control (3.37 $\pm$ 0.14) on day 28 (Fig. 2).

3.4. Measurement of pain threshold

The pain threshold of the paw in the FCA administered rats decreased progressively till day 12. MEPQ (400 and 200 mg/kg) significantly ($P < 0.001$) increased the pain threshold from day 20 and day 24 respectively, whereas MEPQ (100 mg/kg) was less effective, it significantly ($P < 0.01$) increased the pain threshold on day 28. Diclofenac (5 mg/kg), used as standard, significantly ($P < 0.001$) increased pain threshold form day 20. The pain threshold of MEPB (400 mg/kg; 250 $\pm$ 15.2 and 200 mg/kg; 224 $\pm$ 4.7) was evident as compared to FCA control (145 $\pm$ 7.9) on day 28 (Fig. 3).

3.5. Measurement of thermal hyperalgesia (paw withdrawal latency)

There was significant ($P < 0.001$) decrease in paw withdrawal latency of all the rats treated with FCA compared to healthy control. MEPQ (400 and 200 mg/kg) significantly ($P < 0.01$) increased the paw withdrawal latency from day 20 and day 24 respectively, whereas MEPQ (100 mg/kg) showed very minute effect on day 28, it significantly ($P < 0.05$) increased the paw withdrawal latency on day 28. Diclofenac (5 mg/kg) also caused a significant ($P < 0.001$) increase in paw withdrawal latency from day 20 onwards. The paw withdrawal latency of MEPQ (400 mg/kg; 7.32 $\pm$ 0.25 and 200 mg/kg; 6.17 $\pm$ 0.36) was evident as compared to FCA control (2.90 $\pm$ 0.18) on day 28 (Fig. 4).

3.6. Measurement of mechanical nociceptive threshold

The mechanical threshold was observed to be the lowest on day 12. Administration of MEPQ (400 and 200 mg/kg)

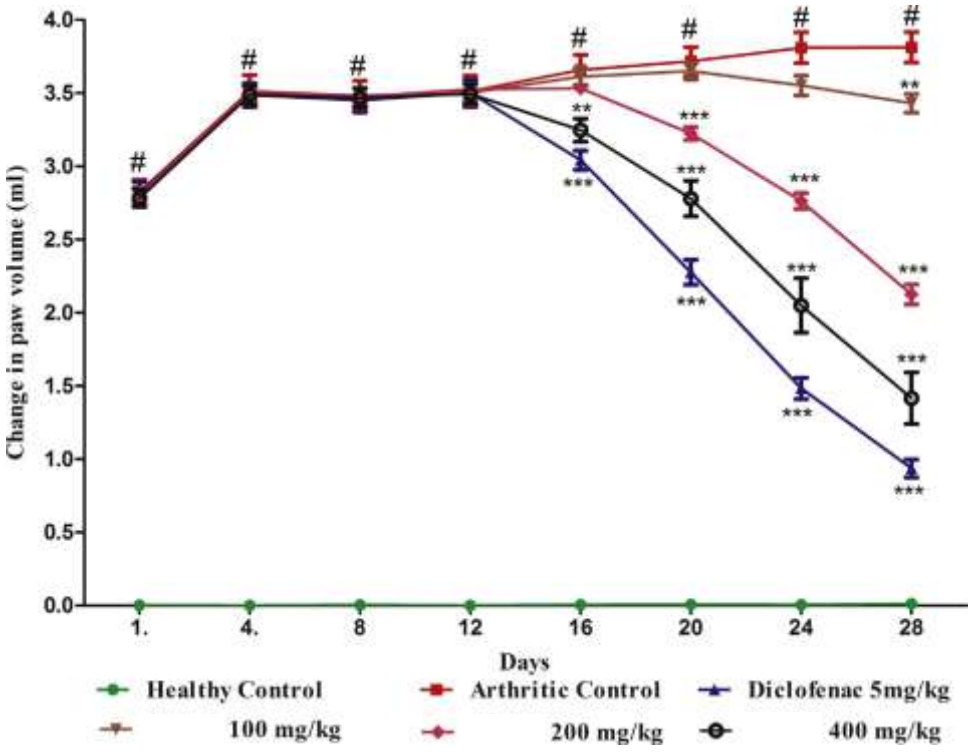

Fig. 1. Effect of MEPB on change in paw volume in FCA- induced arthritis. Values are expressed as mean ±SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

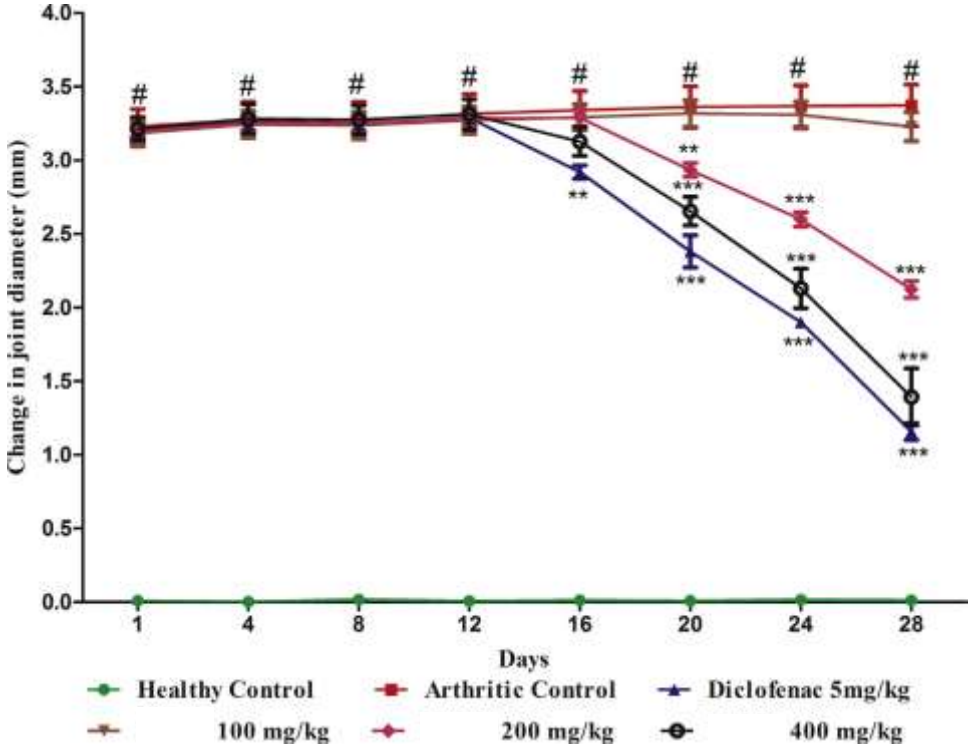

Fig. 2. Effect of MEPQ on change in joint diameter in FCA-induced arthritis. Values are expressed as mean± SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

significantly ($P < 0.001$) improved the mechanical withdrawal threshold from day 20 when compared to arthritic control. How-ever, there was little improvement observed in MEPB 100 mg/kg that significantly ($P < 0.01$) increased mechanical withdrawal threshold on day 28. Diclofenac (5 mg/ kg) showed significant ($P < 0.001$) improvement in mechanical withdrawal threshold from day 16 onwards. The mechanical withdrawal threshold of MEPB (400 mg/kg; 59.82 ± 1.01 and 200 mg/kg; 52.58 ± 1.76) was evident as compared to FCA control (24.97 ± 0.98) on day 28 (Fig. 5).

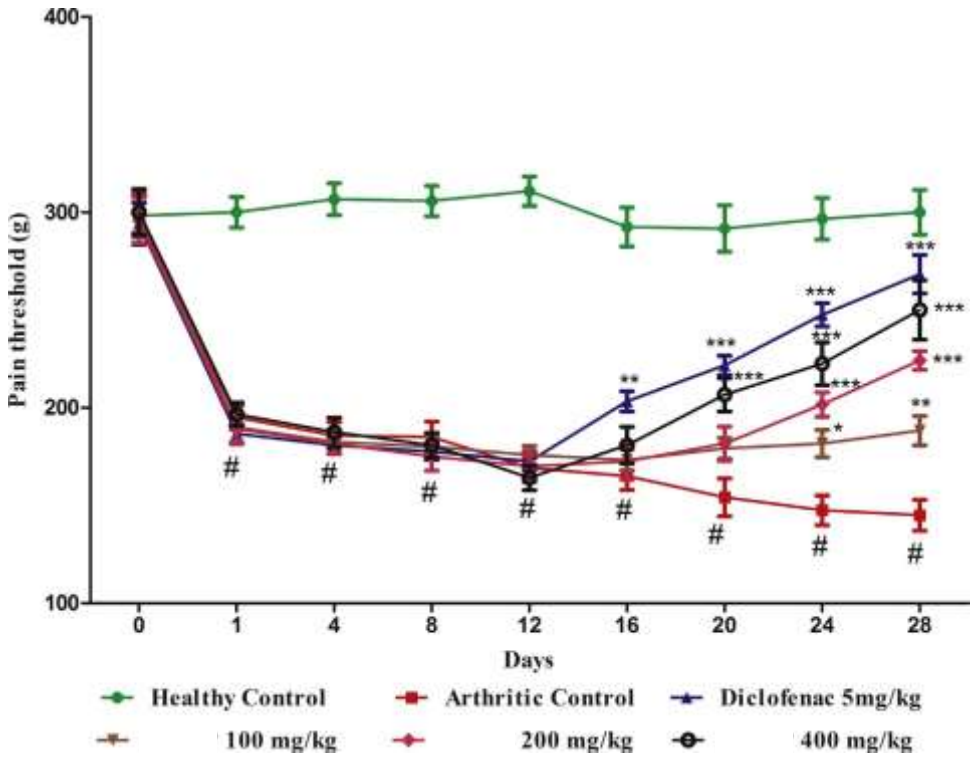

Fig. 3. Effect of MEPQ on pain threshold in FCA-induced arthritis. Values are expressed as mean±SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

3.7. Body weight recording

The rats in the arthritic control group lost body weight as com-pared with the MEPQ and diclofenac treated group. The body weight of MEPB (400 mg/kg; 209 ± 4.10 and 200 mg/kg; 201 ± 5.08) was evident as compared to FCA control group (168 ± 3.50) on day 28. The results indicate that MEPQ (400 and 200 mg/kg) increased the body weight by 23.89% and 19.15% respectively on day 28 while

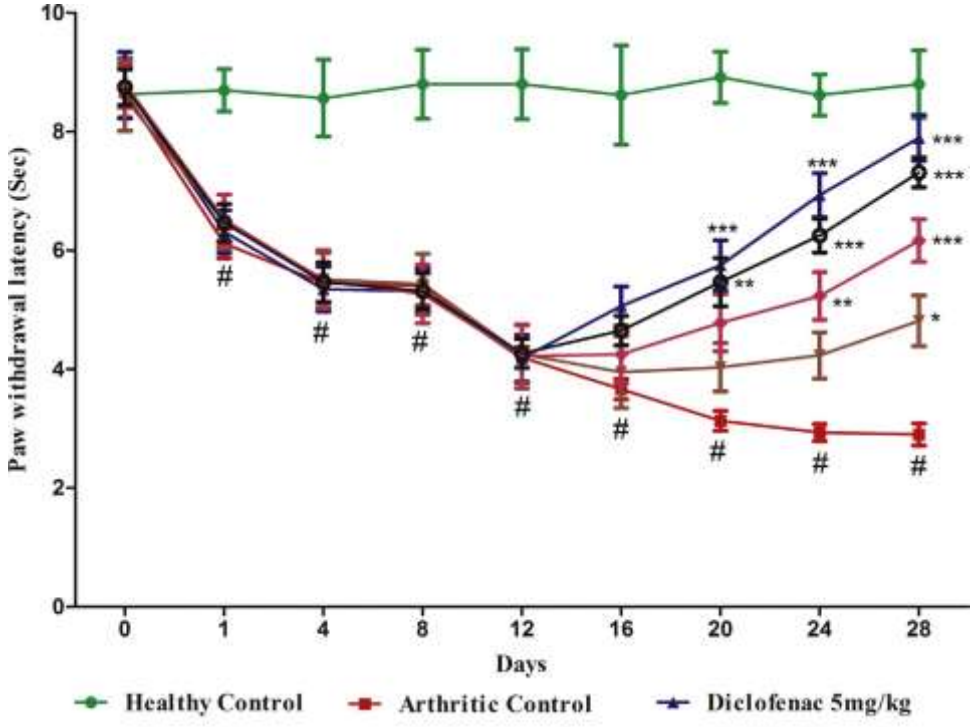

Fig. 4. Effect of MEPQ on thermal hyperalgesia (paw withdrawal latency) in FCA-induced arthritis. Values are expressed as mean ±SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

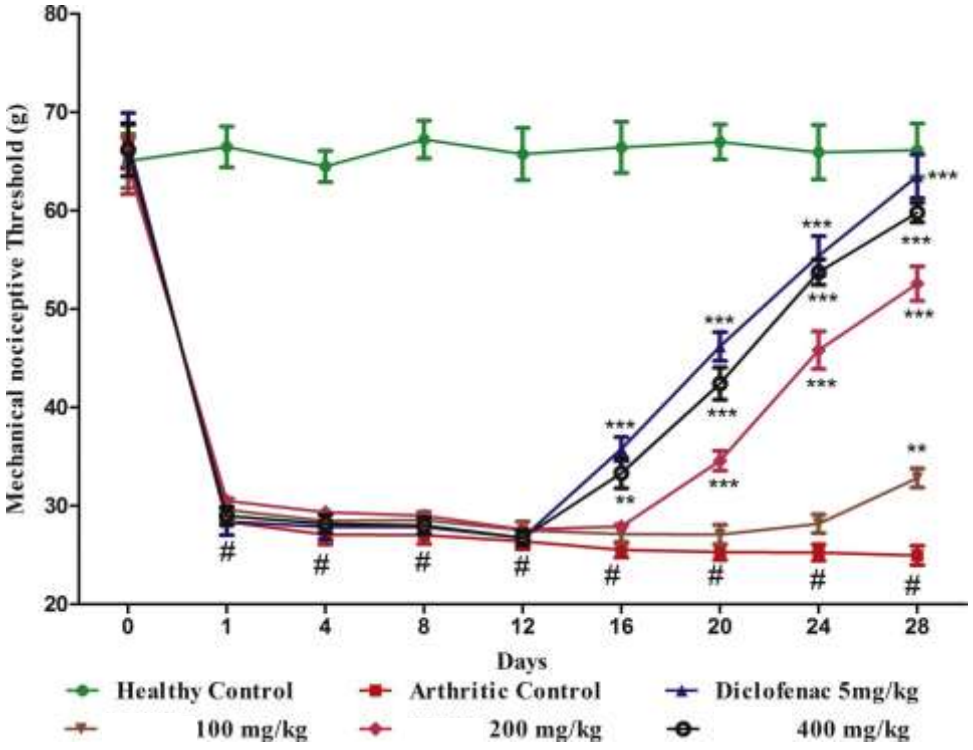

Fig. 5. Effect of MEPQ on mechanical nociceptive threshold in FCA-induced arthritis. Values are expressed as mean SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

diclofenac (5 mg/kg) increased the body weight by 25.37% as compared to arthritic control group.

3.8. Radiological analysis of ankle joints

As shown in Fig. 6, FCA injected rats had developed definite joint space narrowing of the inter-tarsal joints, diffuse soft tissue swelling, cystic enlargement of bone and extensive erosions. FCA control rats suffered from more pronounced bone destruction than MEPQ (400 mg/kg) (Fig. 6F) and diclofenac (5 mg/kg) (Fig. 6C) treated groups. MEPQ (200 mg/kg) showed moderate effect (Fig. 6E), while MEPQ (100 mg/kg) showed no obvious effect (Fig. 6D).

3.9. Haematological and serum parameters

The increased levels of platelets and WBC and decreased levels of RBC and Hb were observed in arthritic control group. These conditions were altered dose dependently by treatment with MEPB and diclofenac. The increased level of serum C-reactive protein and rheumatoid factor observed in arthritic control group were also significantly ($P < 0.001$) decreased by treatment with MEPQ (400 and 200 mg/kg) and diclofenac (Table 1).

3.10. Biochemical parameters

As a result of FCA-induced arthritis, the serum levels of AST, ALT and ALP were increased significantly ($P < 0.001$) and total protein level was decreased significantly ($P < 0.001$) in arthritic group. These enzyme levels were altered by treatment with MEPQ and diclofenac. The level of AST, ALT and ALP were significantly ($P < 0.001$) decreased by treatment with MEPQ 400 mg/kg and diclofenac 10 mg/kg and the level of total protein was significantly ($P < 0.001$) increased. The effects observed by MEPQ were dose dependent (Table 2).

3.11. Anti-oxidant parameters

In arthritic control group significant ($P < 0.001$) reduction in GSH and SOD levels were observed compared to healthy control group. The treatment with MEPQ (400 and 200 mg/kg) significantly ($P < 0.001$ and $P < 0.01$, respectively) increased GSH levels. Further,

Table 1
Effect of MEPB on haematological and serum parameters in FCA-induced arthritis in rats.

Treatment groups	RBC ($^{*}10^6$cells/mm^3)	WBC($^{*}10^3$ cells/mm^3)	Hb(g/dL)	Platelets ($^{*}10^3$ cells/mm^3)	CRP (mg/lit)	RF value (IU/mL)
Healthy control	6.8 ± 0.26	7.3 ± 0.33	14.3 ± 0.22	903 ± 20	1.65 ± 0.14	–
Arthritis control	3.5 ± 0.20$^{\#}$	14.2 ± 0.25$^{\#}$	9.4 ± 0.28$^{\#}$	1734 ± 40$^{\#}$	7.05 ± 0.26$^{\#}$	57 ± 1.20$^{\#}$
Diclofenac (5 mg/kg, per os)	6.1 ± 0.26***	8.4 ± 0.25***	14.1 ± 0.35***	1091 ± 49***	2.88 ± 0.17***	34 ± 0.91***
MEPB (100 mg/kg, per os)	3.8 ± 0.08	13.4 ± 0.64	10.8 ± 0.40*	1626 ± 57	6.03 ± 0.31*	53 ± 1.10*
MEPB (200 mg/kg, per os)	5.0 ± 0.35**	11.5 ± 0.47***	12.2 ± 0.28***	1391 ± 46***	5.10 ± 0.31***	48 ± 1.30***
MEPB (400 mg/kg, per os)	5.8 ± 0.42***	9.6 ± 0.24***	13.2 ± 0.38***	1190 ± 49***	3.92 ± 0.30***	42 ± 0.81***

MEPQ: Aqueous methanolic extract of *Pogostemon quadrifolius* FCA: Freund's complete adjuvant; RBC: red blood cell; WBC: white blood cell; Hb: haemoglobin; CRP: C-reactive protein. RF: rheumatoid factor. Values are expressed as mean SEM for six animals and analysed by one-way ANOVA followed by Dunnett's test, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

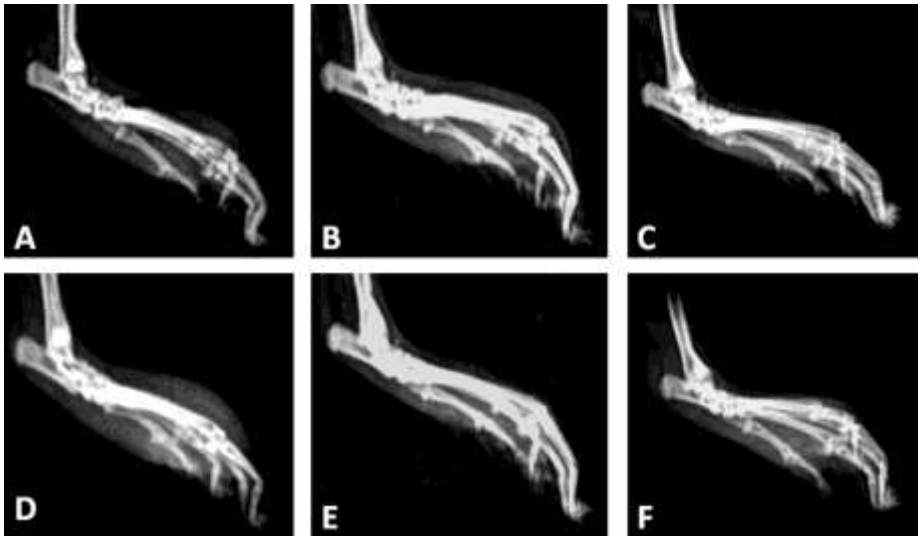

Fig. 6. Radiological analysis of ankle joints. (A) Healthy control. (B) Arthritic control. (C) Diclofenac 5 mg/kg treated. (D) MEPB 100 mg/kg treated. (E) MEPB 200 mg/kg treated. (F) MEPB 400 mg/kg treated.

MEPB (400 and 200 mg/kg) also showed significant ($P < 0.001$) increase in level of SOD.

The MDA levels were significantly ($P < 0.001$) increased in liver of arthritic control group as compared to healthy control group. The treatment with MEPQ(400 and 200 mg/kg) significantly ($P < 0.001$ and $P < 0.01$, respectively) decreased the levels of MDA. The level of MDA in diclofenac treated group also showed significant ($P < 0.001$) reduction (Table 3).

3.12. Histopathological analysis of ankle joints

Histopathology of ankle joint of healthy control rats showed no inflammation, with intact synovial lining and no necrosis of bone (Fig. 7A). FCA treated rats showed massive influx of inflammatory cells, necrosis of bone, chronic inflammation and disturbed synovial lining (Fig. 7B). In contrast to these pathological changes the rats treated with MEPQ (400 mg/kg, per os) and diclofenac (5 mg/kg, per os) showed significant protection against necrosis of bone with low influx of inflammatory cells (Fig. 7F and Fig. 7C, respectively). MEPQ (200 mg/kg, per os) treated rats showed moderate necrosis of bone with little presence of inflammatory cells (Fig. 7E) and MEPB (100 mg/kg, per os) treated rats showed influx of inflammatory cells with evidence of disturbed synovial lining and necrosis of bone (Fig. 7D).

3.13. Measurement of spleen and thymus weights

All the observations were recorded on the last day of the study. MEPQ (400 mg/kg, per os) significantly ($P < 0.001$) reduced the spleen (0.75 ± 0.02) and thymus (0.15 ± 0.01) weights compared to FCA control spleen (0.91 ± 0.03) and thymus (0.21 ± 0.01) weights. MEPB (200 mg/kg, per os) also significantly ($P < 0.05$) reduced the spleen (0.82 ± 0.02) and thymus (0.18 ± 0.01) weights. MEPQ (100 mg/kg, per os) showed no significant results in reducing the spleen and thymus weights. Diclofenac (5 mg/kg, per os) also showed significant ($P < 0.001$) reduction in spleen (0.65 ± 0.02) and thymus (0.12 ± 0.01) weights when compared to FCA control group.

4. Discussion

Alternative medicines for the treatment of rheumatoid arthritis are getting more popular. Many medicinal plants provide relief of symptoms in rheumatoid arthritis whose effects are comparable to that of available conventional medicinal agents [33]. Acute toxicity study revealed the non-toxic nature of the extract at the dose of 2000 mg/kg. Rheumatoid arthritis is a chronic inflammatory disease affecting about 1% of the population in developed countries [34]. Limb swelling, inflammatory cell infiltration, proliferative synovitis, and erosion of the bone and cartilage structure are clinical findings common to human arthritis and adjuvant-induced arthritis rat. Owing to this similarity in pathologic features, the adjuvant-induced arthritis rat is a widely used model of rheumatoid arthritis in evaluating the efficacy of anti-inflammatory drugs [35].

In the present study, MEPQ (400 and 200 mg/kg) treatment showed anti-arthritic effect in all the inflammatory parameters. It significantly decreased the inflammation compared to the arthritic control group as observed by decreased paw volume (Fig. 1) and joint diameter (Fig. 2). The present study revealed that paw volume

Table 2
Effect of MEPQ on biochemical parameters in FCA-induced arthritis in rats.

Treatment groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/dL)
Healthy control	42 ± 2.30	42 ± 1.70	73 ± 3.30	6.6 ± 0.06
Arthritis control	127 ± 4.40$^{\#}$	173 ± 4.80$^{\#}$	475 ± 16.0$^{\#}$	5.1 ± 0.04$^{\#}$
Diclofenac (5 mg/kg, p.o)	59 ± 3.30***	57 ± 2.00***	127 ± 6.70***	6.4 ± 0.04***
MEPQ (100mg/kg, p.o)	110 ± 6.80	162 ± 2.70	444 ± 19.0	5.2 ± 0.02
MEPQ (200mg/kg, p.o)	103 ± 2.60**	124 ± 3.50***	347 ± 14.0***	5.4 ± 0.06**
MEPQ (400mg/kg, p.o)	79 ± 5.50***	63 ± 2.30***	203 ± 5.10***	6.0 ± 0.05***

MEPB: methanolic extract of *Pogostemon benghalensis*; FCA: Freund's complete adjuvant; AST: aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase. Values are expressed as mean SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control $^{\#}P < 0.001$ when compared to healthy control.

Table 3
Effect of MEPQ on anti-oxidant parameters in FCA-induced arthritis in rats.

Treatment groups	SOD (units/mg protein)	MDA (nmol of MDA/mg protein)	GSH (μg GSH/mg protein)
Healthy control	4.80 ± 0.06	2.00 ± 0.03	74.0 ± 2.00
Arthritis control	2.50 ± 0.03$^{\#}$	3.60 ± 0.03$^{\#}$	45.0 ± 1.80$^{\#}$
Diclofenac (5mg/kg, p.o)	3.70 ± 0.03***	2.80 ± 0.04***	64.0 ± 2.20***
MEPQ (100mg/kg, p.o)	2.70 ± 0.04*	3.50 ± 0.04	51.0 ± 2.10
MEPQ (200mg/kg, p.o)	3.00 ± 0.07***	3.40 ± 0.02**	55.0 ± 1.40**
MEPQ (400mg/kg, p.o)	3.30 ± 0.04***	3.20 ± 0.05***	61.0 ± 2.90***

MEPQ methanolic extract of *Pogostemon quadrifolius* FCA: Freund's complete adjuvant; SOD: superoxide dismutase; MDA: malonaldehyde; GSH: glutathione. Values are expressed as mean ±SEM for six animals and analysed by one-way ANOVA followed by Dunnett's test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared to arthritic control $^{\#}$ $P < 0.001$ when compared to healthy control.

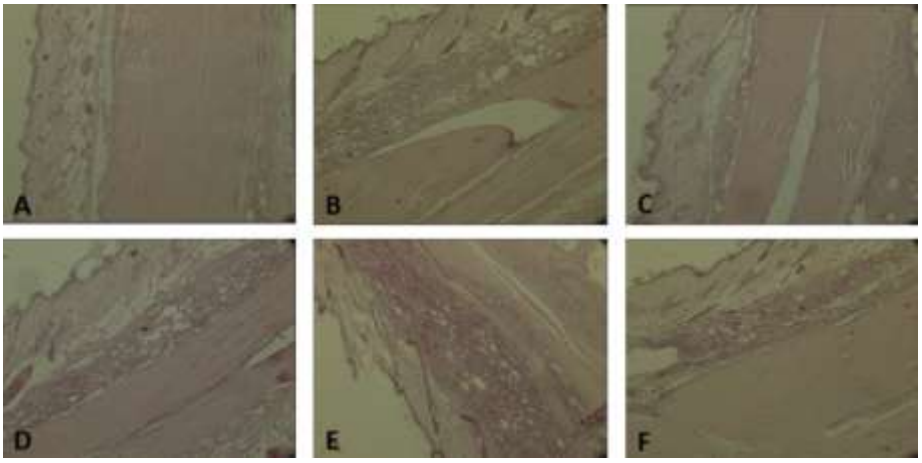

Fig. 7. Histopathological analysis of ankle joints stained with H&E. (A) Healthy control. (B) Arthritic control. (C) Diclofenac 5 mg/kg treated. (D) MEPB 100 mg/kg treated. (E) MEPB 200 mg/kg treated. (F) MEPB 400 mg/kg treated.

and joint diameter increases with ankle stiffness in FCA challenged rats. The analgesic effect of MEPQ (400 and 200 mg/kg) in rats with adjuvant arthritis is also marked as evident by the increase in pain threshold (Fig. 3) thermal hyperalgesia (Fig. 4) and mechanical nociceptive threshold (Fig. 5). The decrease in body weight dur-ing inflammation is due to reduced absorption of nutrients through the intestine [36]. Therefore the restoration of the body weight in rats by MEPQ may involve improvement in the absorption of the nutrients through the intestine of rats (Fig. 8).

It has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1*13* mediated rise in the respective colony stimulating factors and reduction in Hb count in arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and prema-ture destruction of red blood cells [23]. The present study reveals that MEPQ and diclofenac treatments significantly decreased the WBC count and increased the Hb level. In addition to this, other characteristic haematological alterations such as the decreased RBC and increased platelet count were also significantly restored by the MEPQ and diclofenac. MEPQ treatment significantly reduced the levels of RF and CRP. RF could be a marker of RA, characterized by a significant increase in the incidence of distal interphalangeal arthritis [17]. Also a persistent high serum level of CRP is recognized as strong indicator of RA [24].

In the present study, the challenge with FCA (0.1 mL) signifi-cantly ($P < 0.001$) elevated the serum AST, ALT and ALP level and decreased the total protein level. Assessment of the serum levels of AST, ALT and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the drug. The activities of aminotrans-ferases and ALP increases significantly in arthritic rats, since these are good indices of liver and kidney impairment which is also con-sidered a feature of adjuvant arthritis. Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process [27]. The administration of MEPB 400 mg/kg significantly ($P < 0.001$) decreased the level of AST, ALT and ALP and increased the level of total protein that confirms the anti-arthritic activity of the extract.

It is well recognized that free radicals are critically involved in various pathological conditions like cancer, arthritis, inflammation and liver diseases [37]. Lipid peroxidation is a critical mechanism of the injury that occurs during rheumatoid arthritis, which is often measured by analysis of tissue MDA. The large amount of MDA in arthritic control group is consistent with the occurrence of dam-age mediated by free radicals [38]. Treatment with MEPQ (200 and 400 mg/kg) produced a significant reduction of MDA level. GSH reflect the endogenous defence against damage caused by ROS and organic peroxides as they act as an intracellular reductant in oxidation-reduction processes. The decreased levels of GSH in liver of arthritic rats might be due to the excessive consumption of GSH by the system to defend oxidative damage [39]. The production of oxygen free radicals that occurs with the development of arthri-tis leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis [40,41], which is evident by decreased levels of GSH and SOD in arthritic control group. Oral administration of MEPQ to the rats significantly re-established the depleted levels of GSH and SOD, probably by competing for scavenging of free radicals. From the hisopathologi-cal studies of the ankle joint, it is evident that the inflammation of the connective tissue is controlled by treatment with MEPQ (400 and 200 mg/kg). Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis [17]. X-ray studies of the rat paws showed that treatment with diclofenac and MEPQ inhibited the arthritis associated joint alterations. MEPQ (400 and 200 mg/kg) treatment also significantly decreased the spleen and thymus weight compared to FCA control group. The reduction in spleen weight is related to stimulatory effect on the immune system [42].

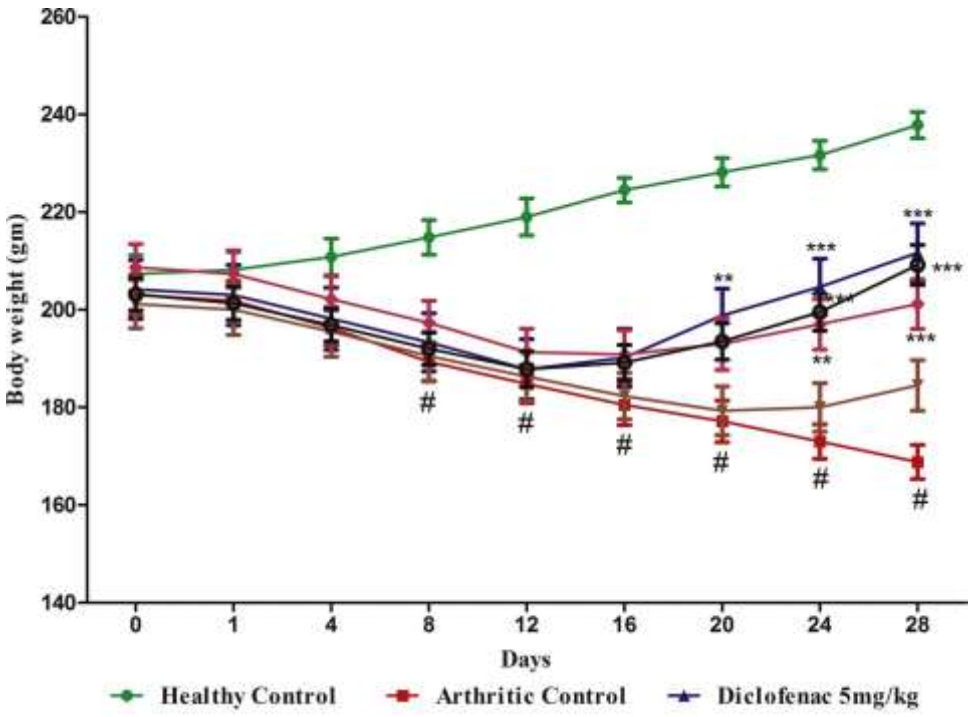

Fig. 8. Effect of MEPQ on body weight in FCA-induced arthritis. Values are expressed as mean ±SEM for six animals and analysed by two-way ANOVA followed by Bonferroni post-hoc test, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to arthritic control #$P < 0.001$ when compared to healthy control.

5. Conclusion

The study revealed that MEPQ (400 and 200 mg/kg) possess anti-arthritic activity that is mediated by its analgesic and anti-inflammatory effects on different parameters evaluated and also through various haematological, biochemical, anti-oxidant, radiological and histopathological parameters. All these results thus predict that MEPQ provide pharmacological rationale for the traditional use of the plant against inflammatory conditions like rheumatoid arthritis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

[1] Zhang R, Fan A, Zhou A, Moudgil K, Zhong M, Lee D, et al. Extract of the Chinese herbal formula *Huo Luo Xiao Ling Dan* inhibited adjuvant arthritis in rats. J Ethnopharmacol 2009;121:366–71.

[2] Tastekin N, Aydogdu N, Dokmeci D, Usta U, Birtane M, Erbas H, et al. Protective effects of l-carnitine and alpha-lipoic acid in rats with adjuvant arthritis. Pharmacol Res 2007;56:303–10.

[3] Liu M, Dong J, Yang Y, Yang X, Xu H. Anti-inflammatory effects of triptolide loaded poly (D L-lactic acid) nanoparticles on adjuvant-induced arthritis in rats. J Ethnopharmacol 2005;97:219–25.

[4] Fan A, Lao L, Zhang R, Zhou A, Wang L, Moudgil K, et al. Effects of an acetone extract of *Boswellia carterii* Birdw (Burseraceae) gum resin on adjuvant-induced arthritis in lewis rats. J Ethnopharmacol 2005;101:104–9.

[5] Geetha T, Varalakshmi P. Anticomplement activity of triterpenes from *Crataeva nurvala* stem bark in adjuvant arthritis in rats. Gen Pharmacol 1999;32:495–7.

[6] Andersen M, Santos E, Seabra M, Silva A, Tufik S. Evaluation of acute and chronic treatments with *Harpagophytum procumbens*on Freund's adjuvant-induced arthritis in rats. J Ethnopharmacol 2004;91:325–30.

[7] Neugebauer V, Han J, Adwanikar H, Fu Y, Guangchen J. Techniques for assessing knee joint pain in arthritis. Mol Pain 2007;3:1–8.

[8] Chillingworth N, Donaldson L. Characterisation of a Freund's complete adjuvant-induced model of chronic arthritis in mice. J Neurosci Methods 2003;128:45–52.

[9] Joshi R. Chemical constituents and antibacterial property of the essential oil of the roots of *Cyathocline purpurea*. J Ethnopharmacol 2013;145:621–5.

[10] Qiang L, Yi M, Lei L, Tai Z. Sesquiterpene Lactones from *Cyathocline purpurea*. Chem J Chinese U 2006;27(5):859–62.

[11] Guoyi M, Li C, Zuqiang L, Andrew H, Martin T. Anticancer activities of sesquiterpene lactones from *Cyathocline purpurea* in vitro. Cancer Chemother Pharmacol 2009;64:143–52.

[12] Nagasampagi B, Sohoni J, Bohlmann F, Zdero C. A Eudesmanolide and a Guaianolide from *Cyathocline purpurea*. Phytochemistry 1981;20(8):2034–6.

[13] Hall I, Starnes C, Lee K, Waddell T. Mode of action of sesquiterpene lactones as anti-inflammatory agents. J Pharm Sci 1980;69(5):537–43.

[14] Joshi A, Baghel V, Pathal A, Tailang M. Phytochemical investigation and medicinal importance of *Cyathocline lyrata*. IJRAP 2010;1(2):302–5.

[15] Mehta A, Sethiya N, Mehta C, Shah G. Anti-arthritis activity of roots of *Hemidesmus indicus* R. Br. (Anantmul) in rats. Asian Pac J Trop Med 2012:130–5.

[16] OECD. Guidelines for testing of chemicals, acute oral toxicity, environmental health and safety monograph series on testing and adjustment N° 425; 2001 [1].

[17] Patel P, Patel D, Patel N. Experimental investigation of anti-rheumatoid activity of *Pleurotus sajorcaju* in adjuvant-induced arthritic rats. Chin J Nat Med 2012;10(4):269–74.

[18] Kumar V, Roy S, Sehgal R, Padhy B. A comparative study on the efficacy of rofecoxib in monoarticular arthritis induced by latex of *Calotropis procera* and Freund's complete adjuvant. Inflammopharmacol 2006;14:17–21.

[19] Lee J, Kim K, Jeong S, Lee S, Park H, Kim N, et al. Anti-inflammatory, anti-nociceptive, and anti-psychiatric effects by the rhizomes of *Alpinia officinarum* on complete Freund's adjuvant-induced arthritis in rats. J Ethnopharmacol 2009;126:258–64.

[20] Banchet G, Boettger M, Fischer N, Gajda M, Brauer R, Schaible H. Experimental arthritis causes tumor necrosis factor-a-dependent infiltration of macrophages

into rat dorsal root ganglia which correlates with pain-related behavior. Pain 2009;145:151–9.

[21] Authier N, Gillet J, Fialip J, Eschalier A, Coudore F. A New Animal Model of Vincristine-Induced Nociceptive Peripheral Neuropathy. Neurotoxicology 2003;24:797–805.

[22] Ramteke V, Tandan S, Kumar D, Devi A, Shukla M, Vellanki R. Increased hyperalgesia by 5-nitro-2, 3-(phenylpropylamino)-benzoic acid (NPPB), a chloride channel blocker in crush injury-induced neuropathic pain in rats. Pharmacol Biochem Behav 2009;91:417–22.

[23] Jalalpure S, Mandavkar Y, Khalure P, Shinde G, Shelar P, Shah A. Antiarthritic activity of various extracts of *Mesua ferrea* Linn. seed. J Ethnopharmacol 2011;138:700–4.

[24] Pepys M, Hirschfield G. C-reactive protein: a critical update. J Clin Invest 2003;11:1805–12.

[25] Mali S, Sinnathambi A, Kapase C, Bodhankar S, Mahadik K. Antiarthritic activity of standardised extract of *Phyllanthus amarus* in Freund's complete adjuvant induced arthritis. Biomed Aging Pathol 2011;1:185–90.

[26] Asquith D, Miller A, McInnes I, Liew F. Animal models of rheumatoid arthritis. Eur J Immunol 2009;39(8):2040–4.

[27] Mythilypriya R, Shanthi P, Sachdanandam P. Salubrious effect of Kalpaamruthaa, a modified indigenous preparation in adjuvant-induced arthritis in rats – A biochemical approach. Chem Biol Interact 2008;173: 148–58.

[28] Misera H, Fridocich. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. J Biol Chem 1972:3170–5.

[29] Slater T, Sawyer B. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fraction in vitro. Biochem J 1971;123:805–14.

[30] Morgon M, Depirre J, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582:67–78.

[31] Patil M, Kandhare A, Bhise S. Anti-arthritic and anti-inflammatory activity of Xanthium srtumarium L. ethanolic extract in Freund's complete adjuvant induced arthritis. Biomed Aging Pathol 2012;2:6–15.

[32] Hu F, Hepburn R, Li Y, Chen M, Radloff E, Daya S. Effect of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. J Ethnopharmacol 2005;100:276–83.

[33] Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discov Today 1999;3:232–8.

[34] Amresh G, Singh P, Rao C. Antinociceptive and antiarthritic activity of *Cissampelos pareira* roots. J Ethnopharmacol 2007;111:531–6.

[35] Noguchi M, Kimoto A, Kobayashi S, Yoshino T, Miyata K, Sasamata M. Effect of celecoxib, a cyclooxygenase-2 inhibitor, on the pathophysiology of adjuvant arthritis in rat. Eur J Pharmacol 2005;513:229–35.

[36] Patil K, Suryavanshi J. Effect of Celastrus paniculatus Willd. seed on adjuvant induced arthritis in rats. Pharmacogn Mag 2007;3:177–81.

[37] Vijayakumar S, Dhanapal R, Sarathchandran I, Saravana A, Vijaya J. Evaluation of anti-oxidant activity of *Ammania baccifera* (L.) whole plant extract in rats. Asian Pac J Trop Biomed 2012:116–9.

[38] Arulmozhi S, Mazumder P, Sathiyanarayanan L, Ashok P. Anti-arthritic and anti-oxidant activity of leaves of *Alstonia scholaris* Linn R. Br. Eur J Integr Med 2011;3:83–90.

[39] Hemshekhar M, Sunitha K, Thushara R, Sebastin M, Shanmuga M, Kemparaju K, et al. Antiarthritic and antiinflammatory propensity of 4-methylesculetin, a coumarin derivative. Biochimie 2013;95:1326–35.

[40] Kizilntuc A, Cogalgil S, Cerrahoglu L. Carnitine and anti-oxidants levels in patients with rheumatoid arthritis. Scand J Rheumatol 1998;27:441–5.

[41] Hassan M, Hadi R, Al-Rawi Z, Padron V, Stohs S. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol 2001;21:69–73.

[42] Pedernera A, Guardia T, Calderón C, Rotelli A, De la Rocha N, Genaro S, et al. Anti-ulcerogenic, and anti-inflammatory activity of the methanolic extract of *Larrea divaricata* Cav. in rat. J Ethnopharmacol 2006;105:415–20.